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Data Article

Data describing expression of formyl peptide receptor 2 in human articular chondrocytes

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ABSTRACT

The formyl peptide receptor 2 (FPR2) belongs to the family of seven-transmembrane G protein-coupled receptors (GPCR) and are expressed by many different cells but mainly studied in immune cells. FPR2 is involved in host defense against bacterial infections and clearance of damaged cells through the oxidative burst and chemotaxis of neutrophils. In addition, FPR2 has also been implicated as an immunomodulator in sterile inflammations, e.g. inflammatory joint diseases. Here we present data regarding FPR2 expression in human articular chondrocytes, isolated from healthy individuals and osteoarthritic patients, on both mRNA and protein level using qPCR and Imagestream flow cytometry. We also present data after receptor stimulation and monitoring of production of nitric oxide, reactive oxygen species, IL-6, IL-8 and MMP-3. The presented data show that human articular chondrocytes from patients with osteoarthritis as well as from healthy individuals express FPR2 both at mRNA and protein

Abbreviations: CL, Chemiluminescence; CT, Cycle threshold; DMEM, Dulbeccoś modified eagle medium; ECM, Extra cellular matrix; FACS, Fluorescence-activated cell sorting; FBS, Fetal bovine serum; FPR, Formyl peptide receptor; GAPDH, Glyceraldehyde 3-phosphate dehydrogenase; GPCR, G protein-coupled receptor; HI, Healthy individual; HRP, Horse radish peroxidase; IL-1β, Interleukin 1 beta; KRG, Krebs Ringer phosphate buffer; MMP, Matrix metalloproteinase; NO, Nitric oxide; OA, Osteoarthritis; PBMC, Peripheral blood mononuclear cells; qPCR, Quantitative polymerase chain reaction; RLU, Relative light units; ROS, Reactive oxygen species.

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level. The biological relevance of FPR2 expression in chondrocytes needs to be further investigated.

Specifications table

Subject	Immunology and microbiology
Specific subject area	Formyl peptide receptor, human articular chondrocytes, inflammation
Type of data	Image Graph Figure Table
How data were acquired	Data was acquired from in vitro experiments using isolated human articular chondrocytes. Instruments: Cell sorter: SH800Z (Sony) qPCR: QIAcube (QIAGEN), NanoDrop Spectrophotometer (Thermo Scientific), ViiA 7 Real-Time PCR (Applied Biosystems). Imagestream: Amnis Imagestream X Mk II (Luminex). Nitrite and reactive oxygen species: CLARIOstar microplate reader (BMG Labtech). ELISA: Spectramax 340PC384 Microplate Reader (Molecular Devices).
Data format	Raw, Analyzed, Processed
Parameters for data collection	Human articular chondrocytes were isolated from cartilage from osteoarthritic patients and individuals without joint disease undergoing hip- or knee prosthesis surgery.
Description of data collection	Human articular chondrocytes were isolated from cartilage using collagenase. Experiments were performed In Vitro. mRNA expression was analyzed with qPCR. Surface protein expression was analyzed with Imagestream flow cytometry. Receptor function was analyzed by monitoring: release of nitric oxide and reactive oxygen species and protein production of MMP-3, IL-6, IL-8.
Data source location	Institution: Department of Rheumatology and Inflammation Research, Institute of Medicine, Sahlgrenska Academy, Gothenburg University. City: Gothenburg Country: Sweden
Data accessibility	Data is hosted with the article. Raw data can be viewed at DOI: http://dx.doi.org/10.17632/y7v5623rt9.1#file-e00a3f3c-cf7d-43f4-a6c3-bb9e5caa77e5 Data files from Imagestream are available upon request by emailing alexander.strid.holmertz@gu.se or karin.onnheim@gu.se

Value of the data

- These data are the first to describe the expression of formyl peptide receptor 2 (FPR2) in human articular chondrocytes.
- These data could benefit research into a variety of different joint diseases such as osteoarthritis and other inflammatory joint diseases.
- These data can be used to further investigate the role of chondrocytes in joint disease, by additional examining of the function of FPR2 in chondrocytes.

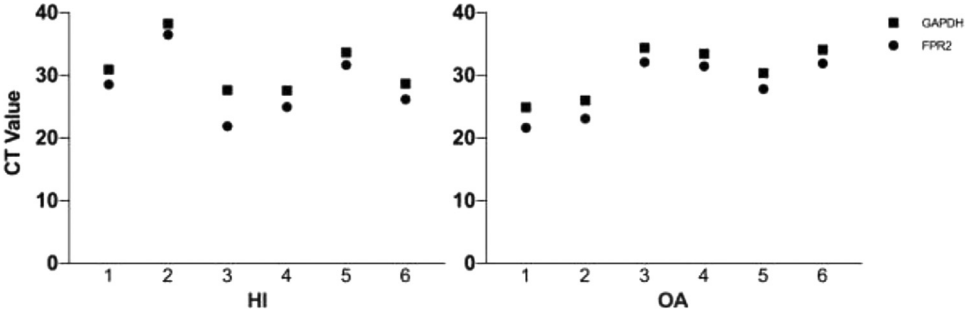


Fig. 1. FPR2 mRNA expression by qPCR with GAPDH as reference gene.

1. Data description

These data describe expression of FPR2 in human articular chondrocytes [1]. Analysis with qPCR shows that chondrocytes from both osteoarthritic patients (OA) and from patients without joint disease (healthy individuals (HI)) express FPR2 on mRNA level (Fig. 1). Imagestream flow cytometry confirmed expression of the protein on the cell surface (Fig. 2). Using specific FPR2 agonist WKYMVm [2] or compound-14 [3], receptor activation was studied in functional assays including release of nitric oxide (NO) measured indirectly as nitrite (Fig. 3) [4], reactive oxygen species (ROS, Fig. 4) [5] and protein production of IL-6 [6], IL-8 [7] and MMP-3 (Fig. 5) [5]. No response from stimulation with FPR2 agonists could be detected when monitoring release of NO or production of IL-6, IL-8 or MMP-3 proteins. Three out of eleven individuals showed a small release of ROS after WKYMVm stimulation.

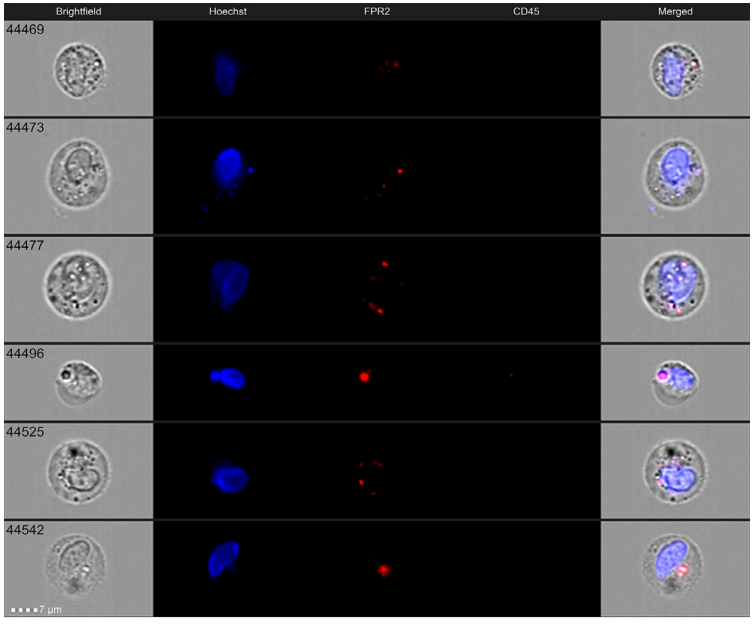
Fig. 6. qPCR for FPR2 was performed on chondrocytes negative for CD45 isolated from HI ($n=6$) and OA ($n=6$) patients. Expression of FPR2 (circle) is presented in relation to reference gene GAPDH (square) for each individual (1–6), and data reported as Cycle Threshold (CT). No statistically significant difference could be detected between OA and HI chondrocytes using non-parametric Mann Whitney *U* test.

Imagestream flow cytometry was performed on chondrocytes and PBMC labeled with FPR2 and CD45 antibodies. Each column shows from left to right: Brightfield, Hoechst, FPR2, CD45 and merged channels. A: Six sequential representative examples of chondrocytes positive for FPR2 from one OA patient. B: PBMC negative (upper row) and positive (lower row) for FPR2. Color should be used when printing this figure. C. Result table showing the individual samples analysed. Total number of cells, positive for FPR2 and procentage. On average, 20% (SD: 14) chondrocytes were $FPR2^{+}$ (OA 16% (SD: 10), HI 25% (SD: 19))

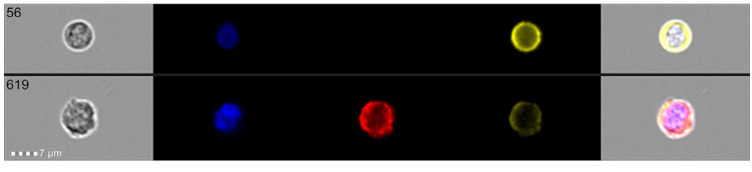
The chondrocyte gating steps (lower row) for CD45 and FPR2 expression was based on PBMC references (upper row). In the first step, unfocused events were removed based on Gradient RMS (image sharpness). Second, beads and doublets were removed by gating for Area and Aspect Ratio. Third, the cells were gated for CD45 expression (removing positive cells from chondrocyte gates, keeping for PBMC). Fourth, the identified cells were gated for bright field view intensity to remove residual ECM before FPR2 expression was analyzed in the final step.

Chondrocytes from OA ($n=6$) patients were cultured with either WKYMVm, IL-1β or with cell medium alone. NO concentration was measured indirectly as nitrite in supernatant after 5 and 24 h of incubation respectively and reported as μM. No significant difference between unstimulated cells and WKYMVm stimulated cells could be detected using the Wilcoxon matched pair signed rank non-parametric test.

The nicotinamide adenine dinucleotide phosphate (NADPH) oxidase activation induced by WKYMVm in isolated chondrocytes was monitored. Baseline chemiluminescence (CL) was measured for 60 s before stimulation with WKYMVm (arrow) and release of ROS was recorded continuously in chondrocytes isolated from OA ($n=9$) and HI ($n=2$) patients. Data is reported as



(a)



(b)

	CHONDROCYTES	FPR2+	FPR2+ (%)
OA1	24983	3578	14,3
OA2	17397	5203	29,9
OA3	20930	2107	10,1
OA4	21747	1717	7,9
HI1	9594	2416	25,2
HI2	2536	164	6,47
HI3	3363	1498	44,5

(c)

Fig. 2. FPR2 expression by Imagestream flow cytometry.

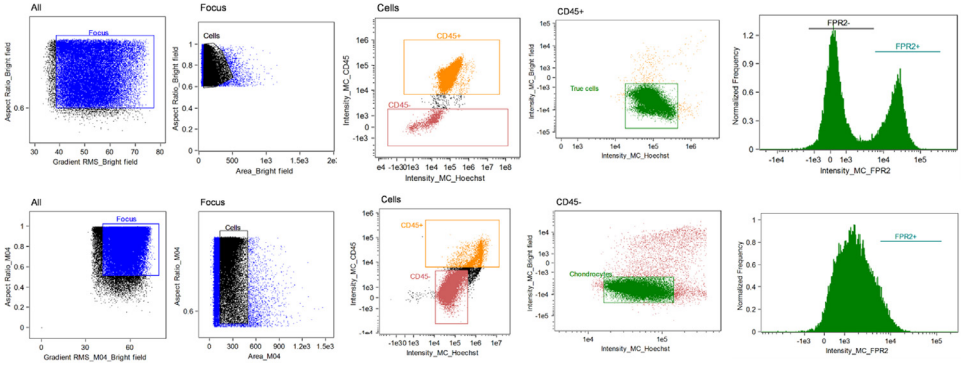


Fig. 3. Imagestream gating strategy.

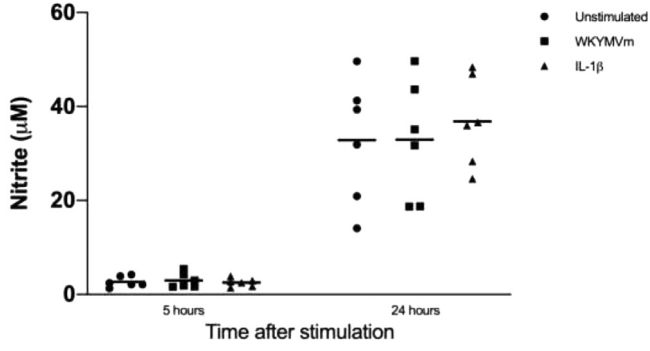


Fig. 4. Production of nitric oxide in chondrocytes.

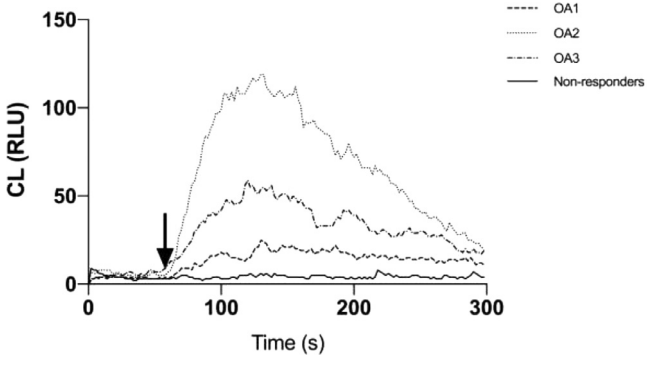


Fig. 5. Production of reactive oxygen species in chondrocytes.

relative light units (RLU) and displayed from responders ($n=3$) and one representative non-responder ($n=8$).

Protein production of MMP-3, IL-6 and IL-8 was measured in supernatants after 48 h of incubation. Chondrocytes isolated from OA patients ($n=3$) were incubated with medium (unstimulated) or stimulated with compound 14 (Comp 14) or IL-1β for 24 h followed by a second stimulation in some samples. No statistically significant differences could be detected using Kruskal–Wallis test by rank, bars indicate mean.

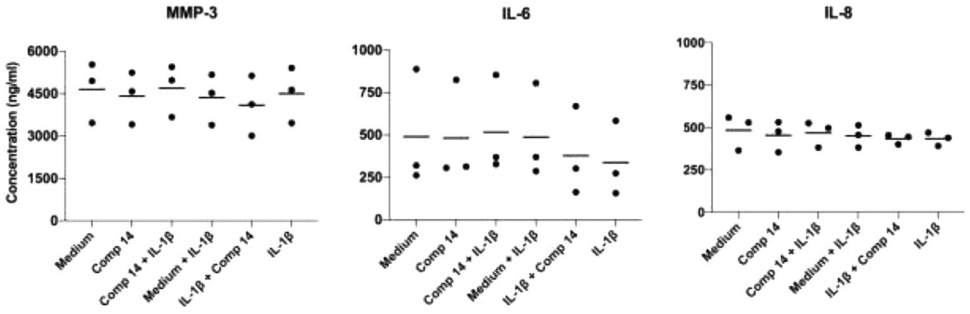


Fig. 6. ELISA analysis for MMP3, IL-6 and IL-8.

2. Experimental design, materials and methods

Chemicals, kits and reagents:

Antibiotics (penicillin/streptomycin).

Cell medium contained: DMEM/F12, 10% fetal bovine serum (FBS), 2% antibiotics and 1% ascorbic acid.

Cell strainer, sterile 40 μm (Fisherbrand, Fisher Scientific)

Collagenase type 2 (Worthington).

Compound 14, kindly provided by Henrik Franzyk (Copenhagen, Denmark) [3].

Dulbeccoś modified eagle medium/nutrient mixture F-12 (DMEM/F12, Gibco).

FACS buffer contained: PBS, EDTA (1 mM), HEPES (10 mM) and 10% FBS.

Fetal bovine serum, heat inactivated (Gibco).

Ficoll-Paque (GE-Healthcare Bio-Science)

High Capacity cDNA Reverse Transcriptase Kit (Applied Biosystems).

Hoechst 33,342 (Invitrogen).

Horse radish peroxidase (HRP, Roche Diagnostics GmbH).

Human IL-1β (Peprotech inc)

Krebs-Ringer Phosphate buffer (KRG) contained: glucose (10 mM), Ca^{2+} (1 mM), Mg^{2+} (1,5 mM), pH 7,3.

Luminol (Sigma-Aldrich).

QIAshredder (QIAGEN).

RLT Buffer (QIAGEN).

RNAse free DNase set (QIAGEN).

RNeasy Mini Kit (QIAGEN).

Trypan Blue (Sigma-Aldrich).

WKYMVM (AltaBioscience)

Probes, antibodies and plates:

qPCR	TaqMan probes (ThermoFisher Scientific). FPR2: FPR2-FAM, Hs00265954_m1 (Catalog no. 435,136). GAPDH: GAPDH-VIC, Hs99999905_m1 (Catalog no. 4,448,485).
Cell sorting	CD45: CD45 APC–H7 (Clone: 2D1, catalog no. 560,178, BD Biosciences). Dilution 1:20.
Imagestream	FPR2: FPRL1 Alexa 647 (Clone 304,405, catalog no. FAB3479R-100UG, R&D Systems). Dilution 1:20 (10 ug/ml). CD45: CD45 APC–H7 (Clone 2D1, catalog no. 560,178, BD Biosciences). Dilution 1:20.
ELISA	MMP3: MMP3 Human Matched Antibody Pair (Catalog no. CHC1543, Invitrogen). IL-6: Human IL-6 DuoSet ELISA (Catalog no. DY206, R&D Systems). IL-8: Human IL-8/CXCL8 DuoSet ELISA (Catalog no. DY208, R&D Systems).

3. Ethics

The collection and use of cartilage from patients were approved by the local ethics committee, Gothenburg (Dnr 334–15, T1075–17, Dnr 2019–04,373). Patients were enrolled following written consent after oral and written information.

4. Collection of cartilage

Cartilage samples were collected from patients undergoing either planned prosthesis surgery (OA, $n = 24$) or acute post traumatic prosthesis surgery (HI, $n = 10$) at the orthopedic clinic, Sahlgrenska University Hospital Mölndal between February 2018 and February 2020. The cartilage samples were collected from femoral heads and knee joints transported in phosphate-buffered-saline (PBS) to the laboratory for preparation. Patients were documented as OA or HI based on hospital files. A description over patients for each experiments is available in the raw data file.

5. Isolation of chondrocytes

Using sterile scalpels cartilage was carved from the sample and cut into small pieces, collected in tubes, and washed with PBS. Cartilage was incubated rotating in 37 °C overnight (16–20 h) in Dulbeccoś modified eagle medium/nutrient mixture F-12 (DMEM/F12), 1% antibiotics, and 0.3% collagenase type 2 to extract chondrocytes from extracellular matrix (ECM). Cells were filtered through a 40 μm sterile cell strainer and washed with PBS containing 2% FBS. Cells were resuspended in fetal bovine serum (FBS) and counted using a Bürker chamber and trypan blue solution staining to estimate viability. Viability was usually estimated at $>$ 95%.

6. Isolation of peripheral blood monocytes

Peripheral blood monocytes (PBMC) were isolated from buffy coats obtained from healthy volunteers using dextran sedimentation and Ficoll-Paque (GE-Healthcare Bio-Science) [8]. After isolation, cells were resuspended in KRG and stored on ice until use.

7. Cell sorting

To avoid contamination of leucocytes from the surgical procedure, cells were sorted based on CD45 negativity prior to qPCR. Frozen samples of isolated cells were thawed and washed with FBS before resuspended in FACS buffer (PBS with 1 mM EDTA, 10 mM HEPES, 10% FBS). Blocking was done with 1% dry milk, 1% FBS and 1% mouse serum for 20 min. Antibody staining was done in FACS buffer with CD45 APC–H7 (BD Biosciences), 1 h on ice. The cells were washed twice before sorting with SH800Z (Sony) based on CD45 negativity using human PBMC as a reference.

8. qPCR

Isolated cells were frozen in 350 μl RLT buffer (QIAGEN), thawed and fully lysed using QIAshredder. The mRNA was prepared using RNeasy Mini Kit in a QIAcube (QIAGEN), with the addition of RNAse free DNase. Extracted mRNA was measured with a NanoDrop Spectrophotometer

(Thermo Scientific). Conversion to cDNA was done with the High Capacity cDNA Reverse Transcriptase Kit. The qPCR assay was performed with 20 ng cDNA per sample set in duplicates on the ViiA 7 Real-Time PCR system (Applied Biosystems) using specific TaqMan probes for FPR2 and Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) as reference gene.

9. Imagestream flow cytometry

Freshly isolated cells were suspended in FACS buffer and blocked with 1% dry milk, 1% FBS and 1% mouse serum for 20 min. Cells were filtered, washed, and resuspended using FACS buffer before staining on ice for 1 h with antibodies specific for CD45 and FPR2 (FPRL1 Alexa 647, R&D Systems). The cells were washed 2 times and resuspended to 1×10^6 cells/25 ml. Hoechst 33,342 (Invitrogen) diluted 1:80 in FACS buffer (2 ml/25 ml cell solution) was added immediately before analysis was performed on the Amnis Imagestream X Mk II (Luminex) and data analyzed using Amnis IDEAS (v. 6.2.64.0, Luminex).

10. Imagestream gating procedure

Imagestream data was analyzed (gated) using Amnis IDEAS. Events (originally 60 000/patient) were included based on image focus (Gradient RMS) as well as area and aspect ratio to remove residual ECM, beads and doublets. Cells were gated based on $CD45^-$ (human PBMC as reference), and chondrocytes were identified from any remaining ECM based on intensity of brightfield images. Chondrocytes were then gated for $FPR2^+$, using human PBMC as reference.

11. Griess assay

Cells (2.5×10^5 cells/ml) were incubated in 200 ul cell medium at 37°C, 5% CO_2 together with 1 μM WKYMVm, 10 ng/ml IL-1β (Peprotech inc) or with cell medium alone. NO was indirectly measured as nitrite in supernatant using the Griess reaction [9] after 5 and 24 h. Briefly, 50 μl of supernatant was mixed with 50 μl of equal parts 1% sulfanilic acid and 0.1% 1-napthtylamine solution and incubated in room temperature for 10 min before absorbance was measured at 540 nm in a CLARIOstar microplate reader (BMG Labtech), a nitrite standard curve (highest concentration 100 μM) was used as reference. Results reported as μM.

12. Chemiluminescence assay

Reactive oxygen species production after activation of NADPH-oxidase through FPR2 stimulation was monitored using a microplate luminol-dependent chemiluminescence assay [10]. Cells (2.5×10^5 cells/ml) were divided into microtiter plate wells (final volume 200 μl) containing Krebs-Ringer Phosphate buffer (KRG), Horse radish peroxidase (HRP) 2 U, and 20 μM luminol (Sigma-Aldrich). Chemiluminescence was monitored, for 1 min prior to and 4 min after stimulation with 0.1 μM WKYMVm (AltaBioscience), in a CLARIOstar microplate reader (BMG Labtech).

13. ELISA

Cells (2.5×10^5 cells/ml, in 200 μl) were incubated for a total of 48 h with either: medium (unstimulated, 48 h), compound 14 (0.1 μM, 48 h), compound 14 followed by 10 ng/ml IL-1β (24 + 24 h), unstimulated followed by IL-1β (24 + 24 h), IL-1β followed by compound 14

(24 + 24 h), or IL-1β alone (48 h). Final concentrations of MMP-3 (Invitrogen), IL-6 (R&D Systems), IL-8 (R&D Systems) protein was measured in cell supernatants with ELISA according to manufacturer's instructions using a Spectramax 340PC384 Microplate Reader (Molecular Devices) and analyzed using SoftMax Pro (v. 5.4.1, Molecular Devices).

14. Statistics

Mann Whitney *U* test was used for qPCR and Imagestream experiments. Non-parametric paired Wilcoxon signed rank test was used for NO measurements. Kruskal–Wallis test by rank was used for ELISA. All statistical analyses were performed in PRISM (v. 8.4, GraphPad) and $\underline{p} \leq \underline{0}.05$ was regarded as significant.

Author contributions

Alexander Strid Holmertz: Study design, acquisition, analysis and interpretation of data. Drafted the work and substantially revised it.

Charlotte Jonsson: Analysis and interpretation of data.

Maziar Mohaddes: Analysis and interpretation of data.

Christina Lundqvist: Analysis and interpretation of data.

Huamei Forsman: Analysis and interpretation of data

Inger Gjertsson: Study design, analysis and interpretation of data. Drafted the work and substantially revised it.

Karin Önnheim: Study concept, study design, acquisition, analysis and interpretation of data. Drafted the work and substantially revised it.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships which have, or could be perceived to have, influenced the work reported in this article.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.dib.2020.105866.

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